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Effect of dietary pH on amino acid utilization by shrimp (*Penaeus vannamei*)

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ABSTRACT

A study was conducted to determine the effect of dietary pH on amino acid utilization by juvenile *Penaeus vannamei*. A basal diet (diet 1), five diets (diets 2–6) supplemented with crystalline amino acids (AA) to simulate the AA pattern of shrimp protein and in which the dietary pH was adjusted from pH 4.8 to 5.0, 6.0, 7.0 and 8.0 ± 0.2, and a 28% shrimp protein control diet (diet 7) were fed to shrimp to satiation 6 times per day for 10 weeks. Growth and feed consumption of shrimp were improved ($P < 0.05$) by supplementing AA and increasing the levels of dietary pH. Maximum weight gain, which was approximately 85% that of shrimp fed the control diet (diet 7), was obtained when the AA diet was adjusted to pH 8.0 (diet 6). However, this growth improvement was due to increased feed consumption rather than to improvement of nutritional value of diets. Shrimp fed diet 7 also had the best feed conversion ($P < 0.05$), which was about two-fold better than those obtained with other diets. There were no significant differences among the survival rates of shrimp fed the various diets.

The pellet water stability at 1 and 3 h was similar for diets 2–6 and diets 1 and 7. However, the dry matter loss of diets 2–6 was approximately twice that of diets 1 and 7. The crude protein losses were negligible for diet 1, 7.0% for diet 7, and 21.2–22.3% for diets 2–6. The total essential amino acids (EAA) including cystine, at 1 h soaking, slightly increased for diets 1 and 7 but decreased by 27.5% for diet 6.

Supplementation of AA and increase in dietary pH had no appreciable effect on moisture and crude protein contents of whole shrimp. Body fat increased ($P < 0.05$) and ash decreased with increasing levels of dietary pH. Shrimp fed the control diet had the highest content of crude protein and fat and the lowest levels of moisture and ash.

This study indicates that growth and feed conversion of *P. vannamei* fed the AA-supplemented diets were inferior to that of shrimp fed the control diet. This may be attributed to the lower water stability of the AA supplemental diets and the apparent loss of AA from these diets. However, improved growth and feed consumption were obtained with increasing pH values of the AA-supplemented diets. This growth improvement was due to increased feed consumption rather than to improvement of nutritional value of the diets.

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INTRODUCTION

Tracer experiments using labeled acetate or glucose have shown that marine shrimp such as *Penaeus japonicus* (Kanazawa and Teshima, 1981), *P. aztecus* (Shewbart et al., 1972, 1973), *P. monodon* (Coloso and Cruz, 1981), and *Palaemon serratus* (Cowey and Forster, 1971) require the same 10 amino acids as fish, rats and pigs. However, the quantitative requirements of the essential amino acids for shrimp are still unknown.

Amino acid test diets consisting entirely of crystalline amino acids or a mixture of intact protein and amino acid are important for the study of protein nutrition. However, it has been reported that juvenile *P. japonicus* (Deshimaru and Kuroki, 1974, 1975; Deshimaru, 1981) and *P. orientalis* (Mai et al., 1988) utilize dietary crystalline amino acids very poorly. Studies at our laboratory to determine the lysine and arginine requirements of *P. vannamei*, using amino acid test diets containing a combination of intact proteins and crystalline amino acids to simulate the amino acid profile of 28% shrimp protein diet, resulted in poor growth and no differences in weight gains between the lysine or arginine levels in the diets. Likewise, it has been demonstrated that common carp (Aoe et al., 1970; Nose et al., 1974) and channel catfish (Dupree and Halver, 1970; Wilson et al., 1977) showed little growth when fed amino acid test diets. However, Nose et al. (1974) reported improved growth response of common carp by adjusting the pH of amino acid diets to 5 or greater. In channel catfish, Wilson et al. (1977) obtained a significant growth improvement by increasing the pH of the amino acid test diets from 4.35 to 6 or 7.

The objective of this study was to determine the effect of dietary pH on the utilization of crystalline amino acids by juvenile *P. vannamei*. Pellet water stability and losses of dietary nutrients after immersion in seawater were also evaluated.

MATERIALS AND METHODS

Seven isocaloric diets (Table 1) were formulated to contain 13 and 28% crude protein (as is basis) for diets 1 and 2–7, respectively. Squid meal and peanut meal served as natural protein sources in diets 1–6, and freeze-dried *P. vannamei* tail muscle was used in the control diet (diet 7). Diets 2–6 were supplemented with a mixture of crystalline L-amino acids (Table 2) to provide an amino acid pattern found in the 28% shrimp protein diet. The diets were maintained isocaloric by adjusting the levels of dextrin and cod liver oil. Cholesterol, lecithin, glucosamine hydrochloride and vitamin and mineral mixes were constant in all diets. Kelvis[1] (Kelco International, San Diego, CA)

[1]Mention of firms or trade products does not imply endorsement by the U.S. Department of Agriculture.

TABLE 1

Percentage composition of experimental diets

Ingredient	Basal diet (diet 1)	pH adjusted diets (diets 2–6)	Control diet (diet 7)
Squid meal	6.00	6.00	—
Peanut meal	20.00	20.00	—
Amino acid mix[1]	—	15.71	—
Freeze-dried shrimp tail muscle	—	—	32.00
Dextrin	36.30	21.30	28.10
Cod liver oil	6.10	6.10	6.50
Cholesterol	0.50	0.50	0.50
Soybean lecithin	1.00	1.00	1.00
Glucosamine-HCl	1.00	1.00	1.00
Binder[2]	3.50	3.50	3.50
Vitamin mix[3]	1.00	1.00	1.00
Mineral mix[4]	7.50	7.50	7.50
Celufil	16.10	15.39	17.90

[1]See Table 2.
[2]Mixture of 2.5% Kelvis and 1.0% sodium hexametaphosphate.
[3]Supplied the following as mg/kg of diet: vitamin A, 80000 IU; vitamin D_3, 2000 IU; vitamin E, 500; vitamin K, 20; niacin, 300; riboflavin, 60; pyridoxine, 57; thiamine, 60; pantothenic acid, 147; biotin, 2; folic acid, 20; vitamin B_{12}, 0.1; choline chloride, 3000; inositol, 200; and ascorbic acid, 500.
[4]Supplied the following as g/kg of diet: modified BT_m salt mixture, 9.771; $CaHPO_4 \cdot 2H_2O$, 35.1; K_2HPO_4, 14.5; NaCl, 10.0; $MgSO_4 \cdot 2H_2O$, 4.0; $CaCO_3$, 1.4; $ZnSO_4 \cdot 7H_2O$, 0.22; KI, 0.007; Na_2SeO_3, 0.001; $CoCl_2$, 0.001.

and sodium hexametaphosphate were used as binders and celufil (U.S. Biochemical Corp., Cleveland, OH) as a filler.

The diets were prepared and stored as described by Lim and Dominy (1990) with some modifications. After all dietary ingredients were thoroughly mixed, 6N NaOH was added to amino acid supplemental diets 3–6 to obtain pH levels of 5.0, 6.0, 7.0 and 8.0 ± 0.2 according to the procedures given by Nose et al. (1974) and Wilson et al. (1977). No pH adjustment was done for diets 1, 2 and 7. The pellets were broken into small pieces and dried in a forced-air Despatch Oven at 50°C for 2.5 h.

Juvenile *P. vannamei* were obtained from the Oceanic Institute, Waimanalo, Hawaii and acclimated for 2 weeks. During this period, they were fed a commercial shrimp feed twice daily. After acclimatization, shrimp of 0.82 to 1.23 g size (average 0.98 ± 0.03 g) were selected, individually weighed and stocked at a density of 15 shrimp in each of 28 flow-through (0.85 l/min) 55-liter glass aquaria. Each aquarium, filled with 52 liters of sand-gravel-filtered seawater, was provided with a tight-fitting lid, plastic netting shelter, and continuous aeration. Any shrimp which died within 72 h after stocking was replaced by a shrimp of similar size.

Each test diet was fed to shrimp in four replicate aquaria 6 times daily to

TABLE 2

Composition of amino acid mixture

L-Amino acid	g/100 g diet
Alanine	0.940
Arginine-HCl	2.053
Aspartic acid	1.570
Cystine	0.177
Glutamic acid	1.970
Glycine	1.164
Histidine	0.255
Isoleucine	0.673
Leucine	0.981
Lysine-HCl	1.762
Methionine	0.549
Phenylalanine	0.454
Proline	0.855
Serine	0.383
Threonine	0.679
Tryptophan	0.134
Tyrosine	0.397
Valine	0.714

satiation for 10 weeks. Feeds were offered 3 times in the morning between 08.00 and 10.00 h and 3 times in the afternoon between 14.00 and 16.00 h. The quantity of feed consumed per aquarium was determined daily.

All aquaria were cleaned daily by siphoning accumulated waste materials and exuviae. Water flow rates were checked and adjusted daily to insure proper water exchange rate. Water temperature, salinity, dissolved oxygen and pH were measured in four randomly selected aquaria twice per week. Water temperature ranged from 25.0 to 28.1 °C with an average of 26.5 ± 0.7 °C, salinity varied from 30.0 to 32.0 ppt with an average of 30.8 ± 0.5 ppt, dissolved oxygen ranged from 5.6 to 8.6 mg/l with an average of 6.4 ± 0.7 mg/l, and the pH values ranged from 7.2 to 7.8 with an average of 7.5 ± 0.2.

Every 14 days, the shrimp in each aquarium were counted and weighed. When the shrimp were removed for weighing, the aquaria were cleaned thoroughly and drained. On sampling days, shrimp were fed once in the afternoon with 60% the amount of feed consumed the previous day to minimize cannibalism.

Water stability of pellets was determined at 1 and 3 h, following the method of Dominy and Lim (1991). Moisture, crude protein and crude fat of diets before soaking (0 h) and after 1 h soaking in seawater at room temperature were determined using AOAC methods (1980). Amino acid contents were determined for diets 1, 6 and 7 before and after 1 h soaking by a private laboratory. All analyses were performed in triplicate.

At the conclusion of the experiment, shrimp were collected and stored frozen at $-30°C$ for subsequent determination of whole body composition. Chemical analyses were done in triplicate following the same methods previously described.

All data, except for the diet proximate and amino acid composition, were subjected to analyses of variance and Dunnett's test to determine the differences between the treatment means ($P<0.05$) (Steele and Torrie, 1960).

RESULTS

The average final weight gain, dry matter feed intake, feed conversion and survival rate of shrimp fed the different diets are presented in Table 3. Shrimp fed the basal diet (diet 1) had the lowest mean weight gain which was lower ($P<0.05$) than those of shrimp in all other treatments. Supplementation of crystalline amino acids to the basal diet significantly improved the growth of shrimp. There was a trend of increased growth with increasing dietary pH, with shrimp fed the diet having a pH of 8 being heavier ($P<0.05$) than shrimp in all other diets with lower pH (diets 2–5). Shrimp fed the control diet (diet 7) had the highest weight gain ($P<0.05$).

Total feed intake data (dry matter basis) reflected the weight gain data for diets 1–6. Because of this, the feed conversion values (g dry feed fed/g wet weight gain) for the groups fed diets 1–6 were not different. On the other hand, because feed consumption of shrimp fed the control diet was less and the weight gain more, the resulting feed conversion was approximately half that of all other groups. Survival rates were generally high and ranged from 83.3% for diet 7 to 96.7% for diets 1 and 6. No significant differences were found among the survival rates of shrimp receiving the various dietary treatments.

Pellet water stability of the diets measured at 1 and 3 h are given in Table

TABLE 3

Weight gain, feed intake, feed conversion and survival of shrimp fed different diets[1]

Diet	pH (± 0.2)	Weight gain (g)	DM feed intake (g/shrimp)	FCR (feed fed/ weight gain)	Survival (%)
1 (Basal)	6.7	1.73^{a}	6.02^{a}	3.47^{a}	96.67
2 (+AA)	4.8	2.47^{b}	7.93^{b}	3.21^{a}	91.67
3 (+AA)	5.0	2.53^{b}	7.89^{b}	3.12^{a}	88.34
4 (+AA)	6.0	3.33^{c}	10.46^{c}	3.14^{a}	90.00
5 (+AA)	7.0	4.05^{c}	12.77^{d}	3.18^{a}	93.33
6 (+AA)	8.0	4.79^{d}	15.98^{e}	3.34^{a}	96.67
7 (Control)	7.0	5.62^{e}	8.89^{bc}	1.60^{b}	83.34

[1]Values reported are means of 4 replicates. Means in the same column having the same superscript are not significantly different at $P>0.05$.

4. The percentage of dry matter remaining after 1 or 3 h of submersion in aerated seawater at room temperature was nearly the same for diets 1 and 7. The amino-acid-supplemented diets with pH adjustment (diets 2–6) had similar water stability which was lower ($P<0.05$) than those of diets 1 and 7.

Dry matter percentages of crude protein and crude fat of test diets before (0 h) and after 1 h submersion in seawater are given in Table 5. The percentage of crude protein in all diets decreased after 1 h submersion in seawater. The percentage of protein loss was only 0.7% for the basal diet (diet 1) and 7.0% for the control diet (diet 7). Diets 2–6 lost considerably higher amounts of crude protein (20.2–22.3%). The apparent level of crude fat after 1 h of soaking was inversely related to the pellet water stability. Diets 2–6, which

TABLE 4

Water stability of experimental diets[1]

Diet	pH (±0.2)	Pellet water stability (%) at:	
		1 h	3 h
1 (Basal)	6.7	91.45[a]	84.74[a]
2 (+AA)	4.8	79.79[b]	70.84[b]
3 (+AA)	5.0	79.43[b]	71.15[b]
4 (+AA)	6.0	78.35[b]	71.39[b]
5 (+AA)	7.0	80.13[b]	70.81[b]
6 (+AA)	8.0	78.81[b]	71.16[b]
7 (Control)	7.0	92.10[a]	85.59[a]

[1]Values reported are means of 3 replicates. Means in the same column having the same superscript are not significantly different at $P>0.05$.

TABLE 5

Percentages of crude protein and fat (dry matter basis) of experimental diets before (0 h) and after (1 h) immersion in seawater[1]

Diet	pH (±0.2)	Crude protein		Crude fat	
		0 h	1 h	0 h	1 h
1 (Basal)	6.7	16.88	16.77 (−0.7)	7.78	8.89 (+14.3)
2 (+AA)	4.8	32.09	25.18 (−21.5)	8.17	10.52 (+28.8)
3 (+AA)	5.0	31.92	25.48 (−20.2)	8.23	10.55 (+28.2)
4 (+AA)	6.2	31.79	24.77 (−22.1)	8.33	10.66 (+28.0)
5 (+AA)	7.0	31.83	25.11 (−21.1)	8.39	10.37 (+23.6)
6 (+AA)	8.0	31.54	24.52 (−22.3)	8.65	10.22 (+18.5)
7 (Control	7.0	32.03	29.79 (−7.0)	8.03	8.93 (+11.2)

[1]Values reported are means of 3 replicates. Numbers in parentheses represent percentages of nutrient gain or loss.

TABLE 6

Essential amino acid content (% dry matter) of experimental diets before (0 h) and after (1 h) immersion in seawater[1]

Amino acid	Diet 1 (basal)		Diet 6 (+AA)		Diet 7 (Control)	
	0 h	1 h	0 h	1 h	0 h	1 h
Arginine	1.32	1.34	2.74	2.19 (−20.1)	2.39	2.21 (−11.3)
Cystine	0.13	0.14	0.28	0.26 (−7.1)	0.23	0.27 (+17.4)
Histidine	0.39	0.42	0.62	0.59 (−4.8)	0.62	0.68 (+9.7)
Isoleucine	0.58	0.56	1.15	1.00 (−13.0)	1.16	1.23 (+6.0)
Leucine	1.08	1.06	1.86	1.51 (−18.8)	2.07	2.13 (+3.0)
Lysine	0.66	0.65	1.82	1.32 (−27.5)	2.05	2.25 0(+9.8)
Methionine	0.20	0.22	0.79	0.37 (−53.2)	0.83	0.77 (−7.2)
Phenylalanine	0.68	0.68	1.11	0.97 (−12.6)	1.16	1.16
Threonine	0.52	0.50	1.21	0.87 (−28.1)	1.10	1.21 (+10.0)
Tryptophan	0.19	0.20	0.32	0.28(−12.5)	0.34	0.37 (+8.8)
Valine	0.64	0.65	1.28	0.95 (−25.8)	1.21	1.23 (+1.6)
Total	6.39	6.42	13.18	10.35 (−26.5)	13.16	13.42 (+2.0)

[1]Values reported are means of 3 replicates. Numbers in parentheses represent percentage gain or loss.

TABLE 7

Whole body percentage composition (dry matter) of shrimp fed various experimental diets[1]

Diet	pH (±0.2)	Moisture	Crude protein	Crude fat	Ash
1 (Basal)	6.7	78.01^{a}	73.53^{a}	5.76^{a}	15.76^{a}
2 (+AA)	4.8	76.39^{abcd}	75.07^{ab}	5.56^{a}	15.31^{a}
3 (+AA)	5.0	77.76^{ac}	74.53^{a}	6.11^{b}	14.99^{a}
4 (+AA)	6.0	77.17^{abc}	74.96^{ab}	6.30^{bc}	13.85^{b}
5 (+AA)	7.0	75.57^{bd}	72.47^{a}	6.53^{cd}	13.64^{b}
6 (+AA)	8.0	76.06^{cd}	73.57^{a}	6.38^{bcd}	13.44^{b}
7 (Control)	7.0	74.58^{d}	77.37^{b}	6.58^{d}	13.39^{b}

[1]Values reported are means of 3 replicates. Means in the same column having the same superscript are not significantly different at $P > 0.05$.

had the poorest water stability, had a greater increase in fat than diets 1 and 7.

Dry matter percentages of essential amino acids (including cystine) of diets 1, 6 and 7 before (0 h) and after 1 h soaking in seawater are presented in Table 6. There were no differences in total essential amino acid content after 1 h submersion for diets 1 and 7, but amino acid content decreased considerably in diet 6 (26.5%) ranging from 4.8% for histidine to 53.2% for methionine.

Whole body composition of shrimp expressed as percent dry matter is given in Table 7. Body moisture content was highest for shrimp fed the basal diet (diet 1) and tended to decrease with increasing dietary pH, becoming significantly ($P<0.05$) lower than diet 1 at pH 7 and 8. On the other hand, body fat tended to increase with increasing pH, becoming significantly higher at pH 5 and above. Carcass protein values did not appear to be related to dietary pH. Ash followed a similar trend as body moisture, decreasing with increasing pH, becoming significant ($P<0.05$) at pH 6 and above.

DISCUSSION

Supplementation of crystalline amino acids to the basal diet and/or increasing dietary pH levels did not affect the survival rate but significantly improved the growth performance of shrimp. Cowey and Sargent (1979) also reported that finfish fed diets supplemented with mixture of 5 or more amino acids grew significantly better than those fed diets supplemented with 3 or less amino acids. They indicated that this growth enhancement was due to rapid and simultaneous absorption of all or many free amino acids which then reach the sites of protein synthesis at about the same time. Frequent feeding (6 times daily) may have also contributed to the increase in growth rate because with frequent feeding, free amino acids from a given feeding may be available more or less simultaneously with amino acids derived from digestion of intact protein, thus resulting in better balance of amino acids. Mai et al. (1988) reported that frequent feeding improved the utilization ratio of free amino acids in *P. orientalis*.

Highest weight gain, which was approximately 85% of that observed for shrimp fed the shrimp protein control diet, was obtained with the amino acid test diet adjusted to pH 8. However, this growth improvement was due to increased feed consumption rather than to improvement of nutritional value of the diets. Feed efficiency values were nearly the same for the basal and amino acid test diets and were only about 50% of that obtained with the control diet. Nose et al. (1974) reported a growth improvement of common carp by adjusting the pH of the amino acid test diets with sodium hydroxide to 5 or higher. However, the growth was only about 60% and feed efficiency did not exceed 20% of those fed the casein control diet. Wilson et al. (1977) found that it was necessary to adjust the pH of the amino acid diets to 6 or 7 for channel catfish to improve growth and feed conversion, although these parameters remained inferior to those of a whole egg protein control diet.

Akiyama (1991) reported that mixtures of synthetic amino acids are effective attractants for shrimp diets. Moreover, crystalline amino acids are readily absorbed by shrimp since they require no further digestion. Retention time of an amino acid diet in the intestine of common carp has been found to be less than half that of a casein diet (Tanaka et al., 1977). Thus, increased feed consumption of the amino-acid-supplemented diets observed in this study may be due to improvement in diet palatability and faster rate of absorption. Likewise, Murai et al. (1981) observed that fingerling common carp and channel catfish fed diets with crystalline amino acids as a major nitrogen source consumed more feed than those with casein and/or gelatin. They suggested that short retention time of the amino acids may have stimulated feed consumption.

The higher feed consumption rate among shrimp fed diets with higher pH may be due to faster rates of digestion and absorption of amino acids. Previous studies on the proteolytic enzymes in the digestive tract of penaeid shrimp have revealed the absence of peptic enzyme activity (Gates and Travis, 1969; Lee and Lawrence, 1982; Maugle et al., 1982; Lee et al., 1984), but the presence of a broad range of alkaline proteases with optimum pH values of 7.0–9.5 (Gates and Travis, 1969; Maugle et al., 1982; Galgani et al., 1984; Tsai et al., 1986; Honjo et al., 1990). Higher levels of sodium as a result of increasing dietary pH may also be a possible cause for the increased rate of amino acid metabolism. Chiu et al. (1984, 1987) suggested that dietary electrolyte balance (Na+K–Cl) influenced the growth, feed efficiency and amino acid metabolism of rainbow trout fed amino acid test diets. However, Wilson et al. (1985) observed that changes in dietary sodium and chloride levels had no effect on growth, feed conversion or metabolism of amino acids of rainbow trout fed intact protein diets. Murai et al. (1983) concluded that dietary pH and level of dietary electrolytes or a combination of both factors play a certain role in amino acid metabolism of common carp but suggested that further studies are needed to clarify the role of electrolytes on amino acid metabolism.

The poor feed conversion obtained with the amino acid test diets as compared to that of the control diet may be due to loss of amino acids during ingestion, since shrimp are very slow feeders. The loss of total essential amino acids was 26.5% for the amino acid test diet whereas that of the control diet increased by 2.0%. The imbalance of essential amino acids resulting from the loss of individual amino acids, such as 53.2% for methionine and 4.8% for histidine, may also have contributed to the poor utilization of the amino acid test diets. Another possible contributing factor is the apparent inability of shrimp to effectively utilize crystalline amino acids as has been reported for *P. japonicus* (Deshimaru and Kuroki, 1974, 1975; Deshimaru, 1981) and *P. orientalis* (Mai et al., 1988). Deshimaru (1976) reported that the absorption rates of amino acids by *P. japonicus* from crystalline amino acid and casein-albumin test diets were 85.0 and 81.1%, respectively. However, Deshimaru (1981) demonstrated that the rate of assimilation of dietary free arginine into the muscle protein by *P. japonicus* was extremely low (0.6%) compared to that of protein-bound arginine (90.5%). He suggested that the inability of free amino acid to substitute for intact proteins in supporting normal growth of shrimp is probably due to differences in the rate of amino acid absorption.

Likewise, Mai et al. (1988) showed that juvenile *P. orientalis* could not absorb supplemental methionine and lysine simultaneously with protein-bound amino acids.

Increasing the dietary pH levels had no significant effect on body moisture and crude protein content of shrimp. The shrimp fed the intact protein control diet had the lowest moisture and highest protein content. Percentage of body fat seemed to be directly related to the growth rate, whereas ash content was inversely related to the size of shrimp.

Results of this study clearly indicate that the growth response and feed consumption of shrimp can be greatly enhanced by increasing the dietary level of pH to 8. This improvement in feed consumption was probably because of improved diet palatability and faster rate of digestion and absorption of amino acids. However, more studies are needed to elucidate the roles of dietary pH and electrolytes on amino acid metabolism in shrimp.

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